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Low Expression of Erythrocyte Complement Receptor Type 1 in Chronic Hepatitis C Patients

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Primate erythrocyte complement receptor type 1 (CR1) plays an essential role in complement-associated immune complex clearance by transporting complexes to macrophages in the liver and/or spleen. Antibody-bound hepatitis C virus, which consists of immune complexes, is observed in patients with chronic hepatitis C. The aim of this study was to clarify the pathophysiological roles of erythrocyte CR1 in hepatitis C virus-infected individuals. We quantified the expression of erythrocyte CR1 with a fluorescence-activated cell sorter system in 57 chronic hepatitis C and 37 chronic hepatitis B cases and 20 normal volunteers. Complement-bound immune complexes were quantified by means of an enzyme-linked immunosorbent assay using anti-C1q and anti-C3d antibodies. Hepatitis C virus-infected patients showed lower erythrocyte CR1 and higher C3d immune complex levels than volunteers ($P < 0.01$ and $P < 0.05$, respectively). An inverse correlation was observed between the erythrocyte CR1 and C3d immune complex levels in hepatitis C virus infection ($r = -0.300$, $P = 0.032$). The erythrocyte CR1 levels in hepatitis C virus infection were lower in patients with severe liver inflammation, cirrhosis, or hepatocellular carcinoma than in those with mild inflammation, whereas the levels did not differ regardless of the disease stage in hepatitis B virus infection. These findings demonstrate that the expression of erythrocyte CR1 is related to immune complex quantity and the severity of liver disease in hepatitis C virus infection.

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KEY WORDS: hepatitis C virus, immune complex, complement receptor type 1

INTRODUCTION

Hepatitis C virus (HCV) is one of the major causative agents of non-A, non-B hepatitis [Alter et al., 1989], which induces a wide range of chronic liver diseases from histologically normal virus carriers to liver cirrhosis (LC) with hepatocellular carcinoma (HCC). Since the establishment of serological and virological HCV assays, information on clinical aspects of HCV infection has accumulated. Quantitative analysis of serum HCV-RNA demonstrated that HCV-infected chronic active hepatitis (CAH), LC, and HCC patients had higher levels of HCV RNA than asymptomatic HCV carriers, implying that high replicative levels of HCV persist in advanced liver diseases [Hagiwara et al., 1993a]. We showed previously that antibody-bound HCV, which consists of immune complexes (IC), can be found in HCV-infected patients and that the ratio of HCV IC to antibody-free HCV increased with the progression of liver disease [Kanto et al., 1995]. These findings raise the possibility that HCV IC are continuously produced and accumulate in the circulation in parallel with disease progression. Although the relevance of HCV IC in the pathogenesis of HCV-related liver diseases and the fate of HCV IC in vivo are not yet understood, impaired IC clearance might be involved in the IC predominance.

In general, IC can be cleared or subjected to phagocytosis by circulating monocytes and/or polymorphonuclear leukocytes or macrophages in the liver and/or spleen [Schifferli et al., 1986]. IC attachment and subsequent phagocytosis by these effector cells occur by way of complement receptors and/or Fc receptors expressed on them [Frank and Fries, 1991]. Complement receptor type 1 on erythrocytes (E-CR1, CD35) can play essential roles in IC clearance in primates by binding C3b/C4b-associated IC and transporting them to macrophages [Schifferli et al., 1986; Ahearn and Fearon, 1989]. Kinetic studies of complement component-associated IC with low-level CR1 erythrocytes showed that the handling and clearance of IC become defective in vivo [Schifferli et al., 1989; Gibson and Waxman, 1994]. Low

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E-CR1 expression has been reported in autoimmune disorders or viral infection, such as systemic lupus erythematosus (SLE) [Ross et al., 1985; Moldenhauser et al., 1987; Cohen et al., 1992] or human immunodeficiency virus (HIV) infection [Tausk et al., 1986; Madi et al., 1992; Pascual et al., 1994]. Furthermore, patients who exhibit acquired immunodeficiency syndromes (AIDS) have lower E-CR1 levels than asymptomatic HIV carriers, suggesting that low E-CR1 levels depend on the disease activity [Tausk et al., 1986]. It remains to be determined how E-CR1 is expressed in HCV-infected patients and whether E-CR1 is involved in HCV IC clearance and HCV-related liver injury.

To clarify the pathophysiological roles of E-CR1 in HCV infection, we determined the degree of E-CR1 expression in HCV-infected patients and investigated its correlation with the amounts of circulating IC and the stage of liver injury. We also examined hepatitis B virus (HBV)-infected individuals and describe the differences between HCV and HBV infection.

MATERIALS AND METHODS

Patients

Fifty-seven HCV-infected patients were included in this study. They were positive by both second-generation anti-HCV antibodies (anti-HCV) and serum HCV-RNA but were negative for hepatitis B surface antigen (HBsAg). We examined, as controls, 37 HBV-infected patients who were positive for HBsAg but negative for anti-HCV or serum HCV RNA. We also examined 20 adult volunteers who were negative for anti-HCV and HBsAg. All volunteers had no clinical history or symptoms of liver disease, HIV infection, or autoimmune disorders. Anti-HCV was assayed with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Ortho Diagnostic Systems Co., Ltd., Tokyo, Japan), and serum HCV-RNA was assayed as reported previously [Hagiwara et al., 1992]. HBsAg was determined using commercially available enzyme immunoassay kits (Dinabot Co., Ltd., Tokyo, Japan). Histological examination was possible for 34 HCV-infected and 25 HBV-infected patients. Histological diagnosis and histological activity index (HAI) scoring [Knodell et al., 1981] were undertaken for liver tissues obtained under peritoneoscopy or ultrasonography after informed consent had been obtained from the patients. Histological grading and staging of chronic hepatitis were performed according to the system proposed by Ishak et al. [1995]. The remaining 23 patients with HCV infection and 6 with HBV infection had HCC, which was diagnosed on angiographical examination. The 6 asymptomatic HBV carriers were defined as those who had no apparent history of alanine aminotransferase (ALT) elevation before examination. The serum HCV RNA titers were determined by means of competitive reverse transcription and polymerase chain reaction (RT-PCR) as reported previously and were expressed as logarithmically transformed HCV RNA copy numbers [Hagiwara et al., 1993b]. HCV genotyping was performed by the method reported by Okamoto et al. [1992] and expressed according to the nomenclature proposed by Simmonds et al. [1994].

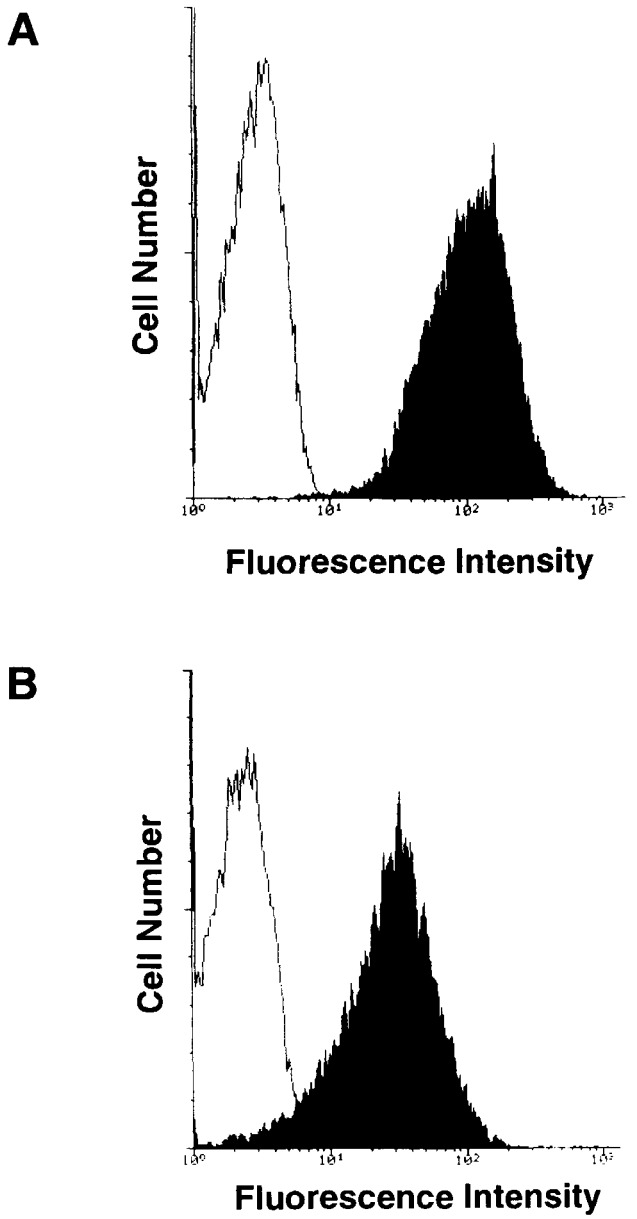

Fig. 1. Histograms of cell numbers and fluorescence intensities of erythrocyte CR1 stained with anti-CR1 antibody (shaded) or mouse purified IgG1 antibody (unshaded) in representative cases. **A:** Erythrocytes recovered from a type C mild chronic active hepatitis patient. **B:** Erythrocytes recovered from a type C severe chronic active hepatitis patient.

Flow Cytometric Analysis of Erythrocyte CR1

Erythrocyte CR1 was analyzed in each patient semiquantitatively using a fluorescence-activated cell sorter (FACS; Becton Dickinson Immunocytometry Institute, San Jose, CA) system as reported previously, with some modifications [Cohen et al., 1987; Spycher et al., 1990; Shapiro et al., 1994]. Erythrocytes were separated by Ficoll-Hypaque density gradient centrifugation. Hepa-

rinized venous blood was diluted with phosphate-buffered saline (PBS) and then layered onto a Ficoll solution with density of 1.119 g/ml (Nadarai Tesque Co., Kyoto, Japan). After the samples had been centrifuged at 720 *g* for 30 minutes, erythrocytes were collected in the bottom layer. They were washed twice with saline, and then 10^6 cells were suspended in 50 μl of PBS + 1% bovine serum albumin + 0.1% sodium azide (PBS + BSA + NaN_3) buffer. Between each staining procedure for E-CR1 described below, washing and suspension of erythrocytes were performed with this PBS + BSA + NaN_3 buffer.

Ten microliters of mouse monoclonal antihuman CR1 antibody (E11 clone, mouse IgG1) (Serotec, Oxford, England) was added to the samples, followed by incubation for 30 minutes at 4°C. Alternatively, as negative controls, 10 μl of mouse purified isotypic antibody (mouse IgG1; Becton Dickinson) was substituted for anti-CR1 antibody. Then, 4 μl of 1:5 diluted biotinylated anti-mouse IgG1 antibody (Zymed Laboratories, San Francisco, CA) was added to 50 μl samples, followed by incubation for 30 minutes at 4°C. Then, 4 μl of phycoerythrin (PE)-conjugated streptavidin (Gibco Biomedical Research Laboratories, Co., Ltd., Gaithersburg, MD) was added to 50 μl of these samples, followed by incubation for 30 minutes at 4°C.

After the samples had been washed, they were fixed with PBS + BSA + NaN_3 containing 4% formaldehyde. Finally, the samples were subjected to FACS analysis within 12 hours after fixation. Under setting the regions of interest on erythrocyte population in a FACS system, the mean fluorescence intensity of stained erythrocytes (MFIs) and that of controls (MFIc) were measured using the Consort 30 software program (Beckton Dickinson). The degree of E-CR1 expression was estimated as the ratio of MFIs to MFIc (MFIs/MFIc) and was expressed as the net fluorescence intensity (NFI). All samples were assayed in duplicate. NFI of E-CR1 in representative cases is shown in Figure 1. In the sample from a mild CAH patient with HCV infection, MFIs and MFIc were 126.61 and 3.32, respectively. Thus, NFI in this patient was determined to be 126.61/3.32 = 38.14 (Fig. 1A). In the same way, NFI in the sample from a severe CAH patient was determined to be 16.34 (Fig. 1B). To confirm the reproducibility of E-CR1 measurements, the fluorescence intensity of PE-conjugated Calibrite Beads (Becton Dickinson) was measured before FACS analysis every time on a separate day. The coefficient of variance (CV) of MFI of Calibrite Beads was less than 5%.

ELISA for C1q- or C3d-Associated Immune Complexes

To quantify circulating C1q- or C3d-associated immune complexes (C1q-IC, C3d-IC) in patients and volunteers, a commercially available ELISA kit employing coated anti-C1q or anti-C3d antibodies (Quidel Co., Ltd., San Diego, CA) was used according to the manufacturer's instructions.

Detection of Viral Genomes in C1q- and C3d-IC by PCR

To determine whether complement-associated IC contain HCV or HBV genomes, IC were recovered from an ELISA plate by treatment with chaotropic ions after termination of the ELISA reaction and subjected to PCR. We used 1.25 mol/liter sodium thiocyanate (NaSCN) to dissociate antigen-antibody complexes through ionic strength [Dandliker et al., 1967]. To recover IC bound to anti-C1q or anti-C3d antibodies coated on a plate, some wells on the plate were washed with PBS and then 1.25 mol/liter NaSCN prepared with 0.2 mol/liter phosphate buffer was poured into the wells. To confirm the recovery of C1q- or C3d-IC, some serum samples were subjected to the antibody-uncoated plate and were treated in the same way. Then, the plate was shaken gently for 60 minutes at room temperature. The solutions in the wells were recovered and stored at −80°C until examination.

Detection of HCV RNA in the recovered samples was performed by RT nested PCR, as reported previously [Kanto et al., 1995]. The primers used for RT nested PCR were designed on the basis of the published nucleotide sequence of the 5′-untranslated region of the HCV genome [Takamizawa et al., 1991]. In brief, extracted HCV RNA was reverse transcribed and then amplified by PCR with external sense (5′-CACTCCCCTGTGAGGAACTACTGTC-3′, positions 29–53) and antisense (5′-ATGGTGCACGGTCTACGAGACCTCC-3′, positions 310–334) primers. Next, nested PCR was performed with the amplified products and internal sense (5′-TGTGAGGAACTACTGTCTTC-3′, positions 37–56) and antisense (5′-ACTCGCAAGCACCCTATCAG-3′, positions 284–303) primers. PCR was carried out for 40 cycles of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 1 minute, followed by a 10 minute extension at 72°C. The oligonucleotide primers used for amplification of HBV DNA by PCR were designed on the basis of the S region nucleotide sequence of the HBV *adr* subtype [Iizuka et al., 1992]. After the samples had been incubated with 10 mmol/liter Tris-HCl, 5 mmol/liter ethylenediaminetetraacetic acid, 0.5% sodium dodecyl sulfate, and 100 μg/ml proteinase K for 3 hours at 70°C, HBV DNA was extracted with phenol/chloroform and purified by ethanol precipitation. The first PCR was carried out with external sense (5′-TCGTGTTACAGGCGGGGTTT-3′, positions 192–211) and antisense (5′-CGAACCACTGAACAAATGGC-3′, positions 685–704) primers for 40 cycles of 94°C for 30 seconds, 55°C for 1 minute, 72°C for 2 minutes, and then 72°C for 10 minutes. The second PCR was performed in the same way as the first PCR with the amplified products and internal sense (5′-CAAGGTATGTTGCCCGTTTG-3′, positions 455–474) and antisense (5′-GGCACTAGTAAACTGAGCCA-3′, positions 668–687) primers. The amplified HCV and HBV PCR products were electrophoresed on 1.5% agarose gel and observed under ultraviolet light.

Assay of Serum Complement and Cryoglobulin

Serum C3 and C4 were assayed by means of turbidimetric immunoassay (TIA). After anti-C3 or -C4 antibodies for TIA (Nittobo Medical, Co., Ltd., Tokyo, Japan) had been added to the serum samples, the turbidity of reactants was measured with a Hitachi 7170 autoanalyzer (Hitachi Industries, Co., Ltd., Tokyo, Japan). Complement activity was determined by Mayer's hemolytic method with sensitized sheep erythrocytes, using a commercial kit (Denka-Seiken, Co., Ltd., Tokyo, Japan). The complement activity was expressed as 50% hemolytic units/ml (CH_{50}). Cryoprecipitates were determined with sera that were stored for up to 7 days at 4°C and centrifuged at 700 *g* for 20 minutes at 4°C.

Statistical Analysis

One-way analysis of variance (ANOVA) and the multiple-comparison Scheffe's test were used to compare ages, complement components, CH_{50}, C1q- or C3d-IC, NFI of E-CR1, ALT, Knodell's HAI scores, and serum HCV RNA titers among groups. Fisher's exact test was employed to compare the distribution of the liver disease status, HCV genotype, and positivity for cryoglobulin in the patient groups. Spearman's correlation coefficient analysis was performed to assess the relationship between NFI of E-CR1 or complements and IC levels. *P* values of less than 0.05 were taken to be statistically significant.

RESULTS

Complement System, C1q-IC, C3d-IC, Cryoglobulin, and E-CR1

In subjects with HBV or HCV infection, the C3, C4, and CH_{50} levels were significantly lower than those in normal adults. Furthermore, the CH_{50} levels in HCV-infected patients were lower than those in HBV-infected individuals (Table I). The amounts of C1q- or C3d-IC were higher in HBV- or HCV-infected patients than in the normal population (Table I). The prevalence of cryoglobulinemia was higher in HCV-infected than in HBV-infected patients (Table I). E-CR1 levels were significantly lower in HCV-infected patients than in normal volunteers (Table I). As is illustrated in Figure 2, HCV RNA and HBV DNA were detected in C1q-IC and C3d-IC recovered from ELISA plates. These results indicated that circulating virus-antibody complexes activated classical and/or alternative pathways of the complement cascade and were bound to complement component fragments. The serum C3, C4, and CH_{50} levels might have decreased because complement components were consumed in these reactions.

Correlation Between E-CR1 and IC Levels

As was described above, HCV-infected patients displayed lower levels of E-CR1 and CH_{50} and higher C3d-IC levels than volunteers. To clarify the relationships between the E-CR1 level and amount of circulating IC, these correlations were examined in HBV- and HCV-infected patients. For HBV infection, no correlation was found between the E-CR1 level and C1q- or C3d-IC ($r = -0.039$, $P = 0.833$, in C1q-IC, and $r = 0.126$, $P = 0.507$, in C3d-IC). In contrast, in HCV infection, E-CR1 was inversely correlated with C3d-IC but not with C1q-IC ($r = -0.300$, $P = 0.034$, in C3d-IC, and $r = -0.144$, $P = 0.304$, in C1q-IC; Fig. 3). These results implied that a low E-CR1 level could lead to the increase of C3d-IC or vice versa.

C1q-IC, C3d-IC, and E-CR1 at Various Stages in HBV- or HCV-Infected Patients

To establish the relationships between the IC level, E-CR1, and liver disease status, we examined these values in patients at various stages of HBV- or HCV-related liver disease (Figs. 4–6). No difference was observed in age between the normal volunteers and each patient group (data not shown).

Among HBV-infected patients, the C1q-IC levels were higher in moderate and severe CAH and LC patients than those in the normal population, whereas they were not different in asymptomatic carriers (AC), mild CAH, and HCC patients. C3d-IC did not differ among the groups, except in AC and mild CAH patients (Fig. 4A). The E-CR1 level did not differ among the groups, regardless of the grade or stage of the liver disease (Fig. 5).

For HCV infection, no difference was observed in the genotype distribution with status of liver disease (Table II). The serum HCV RNA titers in LC and HCC patients were higher than those in histologically normal asymptomatic HCV carriers (AC) and mild CAH patients (Table II). The C1q-IC level was higher in HCC patients, and the C3d-IC levels in all groups were higher than in normal individuals (Fig. 4B). Lower E-CR1 levels were observed in moderate and severe CAH and LC and HCC patients, whereas those in AC and mild CAH patients did not differ (Fig. 6). These results showed that the amounts of C1q-associated HBV-antibody complexes increased in moderate and severe CAH and LC patients and decreased in HCC patients. However, in HCV infection, C3d-associated HCV-antibody complexes were produced even in AC and mild CAH patients, and continued to be present at high levels throughout progression of the liver diseases, which might be related to the lower E-CR1 levels.

Relationships Between IC and Liver Injury

To investigate the roles of IC in HCV-related liver injury, ALT levels and Knodell's HAI scores were compared between patients with C3d-IC levels (≥9.2 mgEq/ml) and patients with low levels (<9.2), who were nominated in the CAH and LC groups. The ALT levels were higher in patients with high C3d-IC levels than in those in low levels (117.5 ± 36.7 vs. 33.1 ± 19.0; $P < 0.05$), whereas the HAI scores were not different between these groups (8.1 ± 4.9 vs 5.4 ± 3.9; $P = 0.13$). In HBV infection, the relationships between the C1q-IC levels and liver injury in CAH and LC patients were also examined. Neither the ALT nor the HAI scores were different between high C1q (≥2.9) and low C1q (<2.9) patients (data

TABLE I. C3, C4, and CH_{50} Levels, C1q- or C3d-Bound Immune Complexes, Cryoglobulin, and Erythrocyte CR1 in Normal Volunteers and HBV- and HCV-Infected Patients†

	Normal (n = 20)	HBV (n = 37)	HCV (n = 57)
Sex (M/F)	12/8	28/9	36/21
Age (years)	52.3 ± 6.8	48.9 ± 10.3	56.4 ± 8.0
Stage of liver disease (AC, mild CAH/moderate, severe CAH/LC/HCC)	—	9/10/12/6	10/12/12/23
C3 (mg/dl) (normal range 61–121)	69.6 ± 7.4	52.4 ± 9.3*[1]	59.7 ± 15.1*[1,3]
C4 (mg/dl) (normal range 18–51)	26.6 ± 7.2	17.9 ± 6.3*[1]	20.9 ± 8.2*[2]
CH_{50} (U/ml) (normal range 30–40)	34.6 ± 3.0	29.4 ± 9.3*[1]	18.2 ± 10.5*[1,3]
C1q-IC (μgEq/ml) (normal <2.9)	0.3 ± 0.7	2.5 ± 3.1*[2]	2.9 ± 5.1
C3d-IC (μgEq/ml) (normal <9.2)	6.1 ± 2.7	8.2 ± 8.3	13.6 ± 9.8*[2]
Cryoglobulin[a]	0/20	2/23	23/49*[1,3]
CR1 (NFI)	28.0 ± 5.9	26.1 ± 5.6	23.1 ± 7.9*[1]

†Values are expressed as means ± SD, AC, asymptomatic HBV or HCV carriers with histologically normal liver and/or normal ALT levels; CAH, chronic active hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; NFI, net fluorescence intensity, representing the ratio of mean fluorescence intensity (MFI) of E-CR1 stained with anti-CR1 antibody to MFI of E-CR1 treated with the purified isotypic antibody.
*[1] $P < 0.01$ vs. normal.
*[2] $P < 0.05$ vs. normal.
*[3] $P < 0.01$ vs. HBV.
[a]Numbers of cryoglobulin-positive cases/numbers of cases examined.

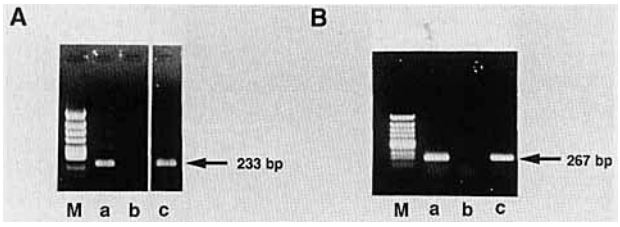

Fig. 2. Detection of HBV DNA (**A**) or HCV RNA (**B**) in C1q- or C3d-immune complexes recovered from an enzyme-linked immunosorbent assay (ELISA) plate after termination of the reaction. M, molecular size marker (ϕ 172 *Hinc*II digest); a, C1q-immune complex; b, sample recovered from the plate that was not coated with anti-C1q and anti-C3d antibodies; c, C3d-immune complex.

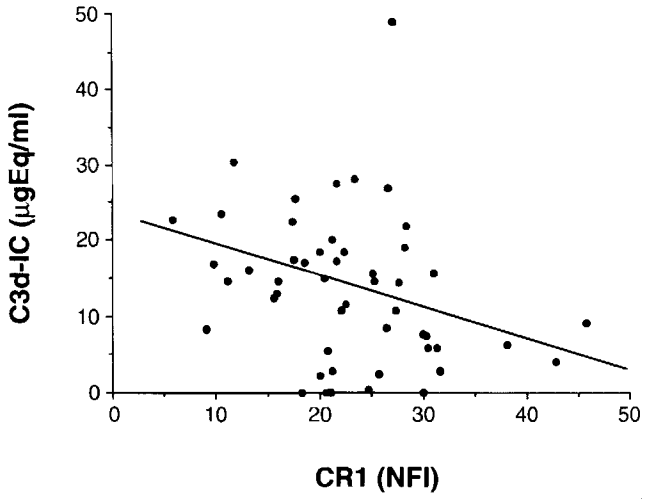

Fig. 3. Correlation between erythrocyte CR1 and C3d-immune complex levels in HCV-infected patients (correlation coefficient = −0.300, n = 50, $P = 0.034$). NFI, see Table I.

not shown). These results indicated that the C3d-IC levels in HCV infection were associated with liver inflammation, whereas the C1q-IC levels were not associated with liver inflammation in HBV.

DISCUSSION

A method is described for estimating E-CR1 expression semiquantitatively by FACS analysis. The number of CR1 on erythrocytes has been reported to be low, ranging from 100 to 1,500/cell [Ross et al., 1985; Cohen et al., 1987]. Thus, a radioimmunoassay (RIA) has been employed until recently to quantify E-CR1. To analyze efficiently E-CR1 levels with a FACS system, we used avidin-biotin conjugation in E-CR1 staining for enhancement of the fluorescence intensities of the stained signals. Some investigators reported good correlation between E-CR1 numbers determined by RIA and E-CR1 fluorescence intensities measured with a FACS system [Cohen et al., 1987; Spycher et al., 1990]. With the procedure presented here, we examined samples from patients and volunteers. Through the activation of complement on IC, C3b/C4b opsonized IC are produced and entered the CR1-mediated clearance pathway. With the aid of Factor I and CR1, circulating C3b/C4b-IC can be readily cleaved into iC3b-IC and subsequently into C3d-IC [Walport and Lachmann, 1993]. These cleaved products are

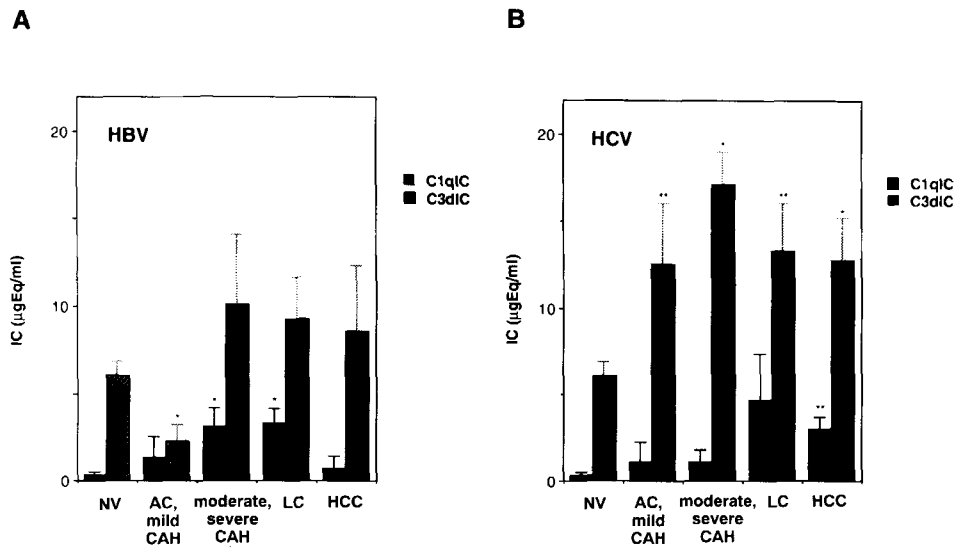

Fig. 4. C1q- and C3d-immune complex levels in HBV (**A**) or HCV infected (**B**) patients. Bars represent means ± S.E.M. NV, AC, CAH, LC, and HCC, see Table I. $*P < 0.01$ vs. normal volunteers, $**P < 0.05$ vs. normal volunteers.

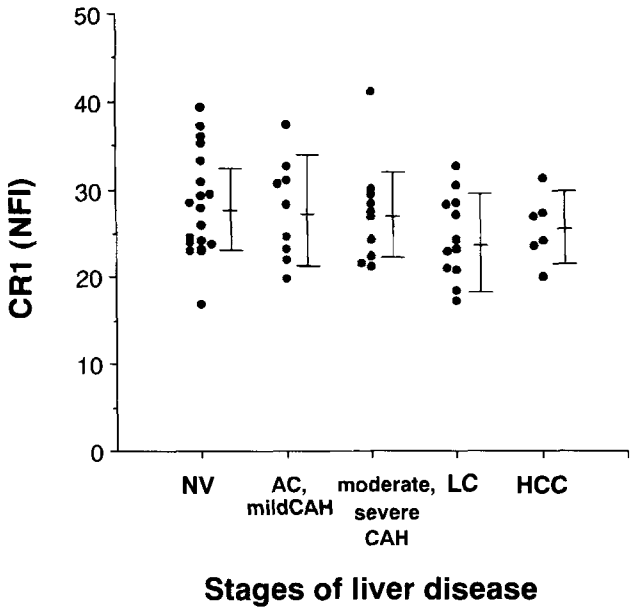

Fig. 5. Erythrocyte CR1 levels in normal volunteers and HBV-infected patients at various stages of liver disease. Vertical bars indicate means ± SD. NFI, see Table I, NV, AC, CAH, LC, and HCC, see Table I.

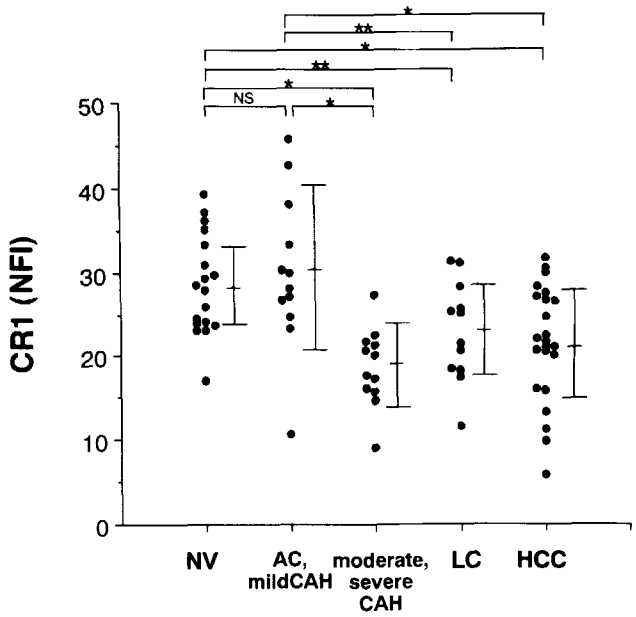

Fig. 6. Erythrocyte CR1 levels in normal volunteers and HCV-infected patients at various stages of liver disease. Vertical bars indicate means ± SD. NFI, see Table I, NV, AC, CAH, LC, and HCC, see Table I. NS, not significant, $*P < 0.01$, $**P < 0.05$

cleared via specific receptors expressed on phagocytic cells [Ross, 1993]. Thus, C3d-IC levels may be correlated with C3b/C4b-IC levels but may be less than these levels. It is demonstrated that the E-CR1 level is lower and that the C3d-IC level is higher in HCV-infected patients than in normal volunteers. In addition, the E-CR1 and C3d-IC levels are inversely correlated. Although it has not been determined whether E-CR1 is involved in HCV IC clearance in vivo, these findings implied that C3b/C4b-associated IC clearance becomes defective owing to a lower E-CR1 level or that the C3d-IC production leads to the E-CR1 decline.

It remains controversial whether the low E-CR1 expression is inherited or acquired. Basically, the extent of E-CR1 expression is determined by genetic inheri-

TABLE II. Serum HCV RNA Titers and HCV Genotypes in Patients With Various Stages of HCV Infection†

	AC, mild CAH (n = 10)	Moderate, severe CAH (n = 12)	LC (n = 12)	HCC (n = 23)
HCVRNA titer[a]	6.4 ± 0.7	7.4 ± 1.1	7.4 ± 0.7*2	7.5 ± 0.6*1
Genotypes (1b/2a/2b/mixed/UC[b])	8/1/1/0/0	9/1/1/0/1	8/2/1/1[c]/1	17/4/1/0/1

†AC, CAH, LC, HCC, see Table I.
[a]Mean ± SD. Titers are expressed as $\log_{10}$ (HCV RNA copies/ml).
*1$P < 0.01$ vs. AC, mild CAH group.
*2$P < 0.05$ vs. AC, mild CAH group.
[b]UC, unclassified.
[c]1b + 2a type.

tance and erythrocyte aging [Moldenhauser et al., 1987; Shapiro et al., 1994]. Low-, moderate, and high-CR1 expressers exist who are detected on restriction fragment length polymorphism (RFLP) analysis of their CR1 complementary DNA [Wilson et al., 1987]. However, in systemic lupus erythamatosus (SLE) or HIV-infected patients, the proportion of genetic inheritance of CR1 expression was not different from that in the healthy population [Moldenhauser et al., 1987], and their E-CR1 levels depended on the disease activity or status, suggesting that such low E-CR1 conditions also arise in an acquired manner [Ross et al., 1985; Tausk et al., 1986]. Although RFLP analyses were not performed in this study, the E-CR1 levels in HCV infection were lower in patients with advanced liver disease than in those with mild or without liver inflammation. This finding implied that a low E-CR1 level in HCV infection is a secondary and not only a genetically determined event.

Then why was low E-CR1 expression observed in HCV but not in HBV infection? Furthermore, it is also unclear why E-CR1 expression in HCV infection was lower in moderate and severe CAH and in LC and HCC patients than in histologically normal virus carriers (AC) and mild CAH patients. Some investigators reported possibilities regarding the induction of secondary lower CR1 expression, such as 1) increasing CR1 degradation by IC or complement activation [Cosio et al., 1990], 2) splitting off of the CR1 structure during circulation [Ross et al., 1985], 3) occupation of the C3b/C4b binding site of CR1 by IC [Edberg et al., 1987], 4) increasing peripheral catabolism or proteolysis [Cohen et al., 1992; Pascual et al., 1994], 5) increasing ligand-specific or -nonspecific CR1 internalization into cells [Turner et al., 1988], or 6) decreasing CR1 biosynthesis. Among these hypotheses, the more plausible explanations for low E-CR1 levels are degradation, splitting, and occupation of CR1.

Through the process of IC removal from E-CR1 by the liver, C3b/C4b-IC are cleaved into iC3b, and the E-CR1 receptor site is simultaneously damaged [Ross, 1993]. Cosio et al. [1990] reported on how IC injection affects E-CR1 levels in vivo. When complement-associated IC were transiently injected into the circulation, the E-CR1 level first decreased and then gradually recovered, and this initial decline required complement activation. However, continuous injection of IC led to a persistent decrease in the E-CR1 level. In the present study, HCV-infected patients had higher C3d-IC levels and lower CH_{50} levels than HBV-infected patients. In addition, HCV-infected LC and HCC patients showed higher C1q-IC and higher serum HCV RNA titers than AC and mild CAH patients. Thus, continuous HCV IC production and subsequent complement activation might induce the destruction or alteration of E-CR1 and persistent low E-CR1 levels. However, the E-CR1 level of AC and mild CAH patients was the same as that of normal volunteers, whereas AC and mild CAH patients showed higher C3d-IC level than volunteers. Therefore, the greater HCV loading on patients may also contribute to the E-CR1 decline. Next, the hyperdynamic circulation or altered erythrocyte membrane structure observed in cirrhotic patients may contribute to splitting off of the extracellular portion of E-CR1. This explanation is applicable to the low E-CR1 levels in LC and HCC patients but not to those in HCV-infected CAH patients. Finally, blocking of binding of anti-CR1 antibodies to E-CR1 by IC occupation may be unlikely, insofar as we used a monoclonal anti-CR1 antibody (E11) that did not compete with complement-associated IC at CR1 [Edberg et al., 1987]. The remaining hypotheses must be investigated further.

Recently, the close association between HCV infection and mixed cryoglobulinemia was reported [Agnello et al., 1992]. Cryoglobulins or IC can be involved in the pathogenesis of extrahepatic syndromes, including vasculitis and glomerulonephritis [Johnson et al., 1993]. However, it is not known whether cryoglobulins, IC, or concomitant complement activation can lead to liver injury. In the present study, HCV-infected patients with higher C3d-IC levels exhibited higher ALT levels than patients with lower C3d-IC levels. To elucidate the pathological roles of IC and the complement system in HCV-related liver injury, the direct detection of the presence of complement components and/or membrane attack complex around injured hepatocytes may be required.

Another immunological potential of IC has been recently reported. Feldman et al. demonstrated that IgG type IC could inhibit interferon (IFN)-γ induced gene expression in vitro [Feldman et al., 1995]. Although it has not been proved that this inhibition can occur in vivo, this finding raises the possibility that circulating

IC themselves impair IFN-γ mediated immunological reactions, such as expression of major histocompatibility complex (MHC) class II gene or induction of cell-mediated cytotoxicity of macrophages and natural killer cells. The involvement of cell-mediated immune response in HCV-related liver injuries is well known [Koziel et al., 1993]. Thus, the IC accumulation in patients can be one of the etiologic factors leading to impairment of cell-mediated cytolysis of HCV-infected cells and subsequent HCV persistence.

In summary, we have demonstrated that low E-CR1 expression was associated with high C3d-IC levels in HCV infection. Although it has not been demonstrated that low E-CR1 levels directly induce IC accumulation in HCV-infected patients, we presented the possibility that low E-CR1 expression is related to defective IC clearance and the persistence of HCV. IC clearance in vivo occurs mostly via C1q, C3b/C4b (CR1), C3d, or Fc receptors. It is necessary to assess how these receptors modulate HCV IC clearance and what proportion the whole IC clearance system in vivo is accounted for by E-CR1-mediated transportation system. Acceleration or augmentation of HCV IC clearance by modulating complements or the complement receptor system could be a therapeutic modality for controlling chronic HCV infection.

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